

THE FLICKER-RESPONSE CONTOUR FOR THE CRAYFISH

II. RETINAL PIGMENT AND THE THEORY OF THE ASYMMETRY OF THE CURVE

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I

The flicker-response contour ($F - \log I$) for the crayfish *Cambarus bartoni* resembles that for other arthropods having markedly convex eyes (see Crozier and Wolf, in press). Only its very uppermost part can be fitted by a probability integral. Over its lower part the slope increases too rapidly, so that the whole curve is quite asymmetrical. This departure from the rule observed in the responses of vertebrates (see Crozier and Wolf, 1937*a* and *b*, 1938*a*) has been accounted for (Crozier and Wolf, 1937*c*, 1938*b*) by the shape of the optic surface in the majority of arthropods. With increasing flash-intensities the retinal area effectively involved is increased, which results in a higher F ; this is due to the greater chance of exciting ommatidia toward the circumference of the curved eye. Confirmation of this view, consistent with the consequences of changing the light-time fraction in the flash-cycle (Crozier and Wolf, 1937*c*, 1938*b*), is given by the fact that an arthropod with sufficiently flat optic surfaces, the isopod *Asellus* (Crozier and Wolf, 1939), gives a flicker-response contour which is a perfectly symmetrical probability integral. The asymmetry of the curve with *Anax* is appropriately reduced by blocking out all but a central area of the eye (Crozier and Wolf, 1937*c*, 1938*b*), and in a form with still more markedly curved optic surfaces (*Cambarus*) (see Crozier and Wolf, in press) the asymmetry is much more extreme.

In our experiments with *Anax* (Crozier and Wolf, 1937*c*, 1938*b*) the limitation of the increase of effective retinal area with increase of illumination by painting portions of the eyes was recognized to be imperfect. A certain amount of leakage of light near the margins of a cap of enamel, and under its edge, cannot be prevented. A neater method of accomplishing the purpose is to use the migrations of retinal pigment cells. The flicker-response contours we have discussed were determined with animals previously dark-adapted. For such a crustacean as *Cambarus* this means that the proximal retinal pigment is below the level of the receptive retinulae, the distal pigment cells well

out toward the surface of the eye around the crystalline cones. The retinulae are completely unshielded from laterally spreading light, and the condition is that for the "superposition" type of eye (Exner, 1891). In the eye well light-adapted the forward migration of the proximal pigment shields the retinulae, while the inward movement of the distal pigment forms around each ommatidium an opaque tube of pigment along the length of the crystalline lens and down to the proximate pigment (Bernhards, 1916; Day, 1911; Parker, 1932). The effective isolation of each recipient unit from light other than that proceeding down the axis of the ommatidium then produces the condition for the "apposition eye" (Exner, 1891).

For our purposes, however, no use could very well be made of the control of retinal pigment migration by light. The process of light adaptation involves not only movements of the retinal pigment cells, but also, it must be presumed, the intrinsic photic adaption of the visual response system itself. At the same time, if some other procedure could be found to cause the retinal melanophores to assume the "light-adapted" condition, it should serve admirably for a test of certain properties of the *Cambarus* flicker-contour. It should also give some direct behavioral evidence as to the functional rôle of the retinal pigment and its movements, as well as providing material for a logical approach to the method of estimating the time-course of visual light-and-dark-adaptation in such animals.

It was pointed out to us by Dr. J. H. Welsh that extracts containing the "eyestalk hormone" from the optic peduncle produce an effect on the melanophores and also on the movement of retinal pigment (Kleinholz, 1934, 1936, 1938; Welsh, 1939) in dark-adapted eyes of *Cambarus*, so that injection of sufficient extract into a dark-adapted animal leads to the migration of retinal pigment into positions characteristic of the normal light-adapted state. This we have verified in *C. bartoni*.

II

The observational procedure was identical with that employed in measuring the flicker-response contour for dark-adapted *Cambarus* (Crozier and Wolf, in press): temperature 21.5°, 50 per cent light-time in the flash cycle. To keep the handling of the animals uniform with respect to time after injection and the like, a lot of 5 rather than of 10 was used. The eyestalks from 10 *Cambarus bartoni* were extracted in Ringer solution. Into each crayfish prepared for observation there was injected into the abdomen 0.08 ml. of extract, the equivalent of 2 eyestalks. After 75 to 90 minutes in the dark the crayfish are

bluish in body color and by means of a beam of light directed into the eye the retinal pigment is seen to be in the position characteristic of light adaptation. Sectioned eyestalks fixed in hot water at this stage show the condition clearly under the ultrapak microscope. In the normal dark-adapted eye the proximal pigment is retracted below the basement membrane, while the distal pigment is out between the crystal cones. There is no detectable pigment between the ommatidial units. After about 90 minutes in darkness subsequent to injection of eye-stalk extract, the proximal pigment surrounds the

TABLE I

Data for the flicker-response contour of the crayfish *Cambarus bartoni*, with eye-pigment in the "light adapted" state as result of injection of eye-stalk hormone. $N = 5$ individuals, $n = 3$ observations on each; the same individuals used throughout; $t^\circ = 21.5^\circ \text{C.}$; $t_L = t_D$. See Fig. 1. I in millilamberts, F in flashes per second. $P.E._1 = P.E.$ of the dispersions.

F	F_m	$P.E._1 F_1$	$\log I_m$	$\log P.E._1 I_1$
2			3.3404	5.9958
5			2.0777	4.3334
8			2.2584	4.4806
12			2.5249	4.8155
16			2.6674	4.9737
20			2.7771	3.0398
25			2.9254	4.8291
30			1.0730	3.2355
35			1.2865	3.6169
40			1.5937	3.8785
42	41.79	0.289	0.00	2.3664
43			0.0233	1.2823
	43.10	0.357	1.0077	
	43.23	0.361	0.50	
	43.66	0.282	1.00	
			1.25	
44			1.5231	0.1464
	44.05	0.130	1.50	

retinulae, while the distal pigment now envelopes each ommatidial unit down to its base. The condition is one of quite complete shielding of each ommatidium by a dense layer of black pigment, more extreme than is the case in ordinary light adaptation.

III

The determinations of mean critical flash-intensity and mean critical flash-frequency for response (Crozier and Wolf, in press) to visual flicker are given in Table I. Comparison with the results for normally

dark-adapted *Cambarus bartoni* (Crozier and Wolf, in press) shows that there is a pronounced (reversible) effect of the injection of eyestalk hormone upon the properties of the flicker-response contour. This cannot reasonably be traced to an effect of the eye-stalk extract upon the intrinsic processes of photic excitability, for several reasons. In

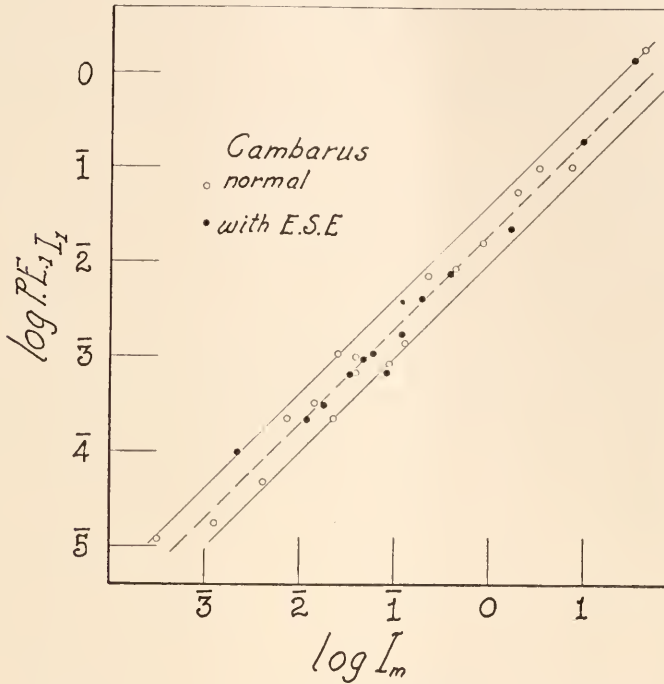


FIG. 1. The variation of I_1 for normal *Cambarus bartoni* and after injection with eye-stalk extract (E.S.E.); Table I; see text.

the first place injection of ca. 0.06 ml. of the eyestalk extract into *Anax* (dragon fly) nymphs produces no detectable effect either on pigment migration or on the flicker-response curve, as the following observations showed (tests on 5 individuals):

F	Normal log I_m	Normal + eye- stalk extract log I_m
20	2.473	2.478
30	2.749	2.745
	2.741	2.750

Any effect of this sort would thus have to be specific. In the second place, the results of adapting *Cambarus* are rapidly apparent even when the retinal pigment is already fully advanced into the "light" position, as subsequently shown (§ IV). Finally the various modifica-

tions of the flicker-response contour are those to be expected as the result of the optical shielding of the ommatidia, so that no specific effect on excitability need be invoked.

For any given level of flash-intensity the variation of I_1 among the individuals used is statistically of the same magnitude as for the normal group previously examined (Crozier and Wolf, in press). The 5 individuals giving the data of Table I were in the lot of 10 providing the normal curve for this species (Crozier and Wolf, in press). The scatter of the variation indices ($P.E._{I_1}$) is even a little less than might have been expected in view of the smaller number of readings in the eyestalk injection series (Fig. 1).

The effects to be expected if the "dark" position of the retinal pigment shields ommatidia from all but light parallel to the reticular axis, and if this is to prevent the recruitment of optic impulses from a larger retinal surface as flash intensity is increased, are the following: (1) the total achievable sensory effect ($= F_{max.}$) must be reduced; (2) at given I , F must be less; (3) the asymmetry of the $F - \log I$ curve must be markedly reduced; and (4) it would not be surprising to find the slope of the "fundamental" curve increased (i.e., $\sigma'_{\log I}$, for the ideal frequency distribution of $\log I$ thresholds, *reduced*), owing to the mechanical exclusion of a large proportion of the otherwise marginally excitable units.

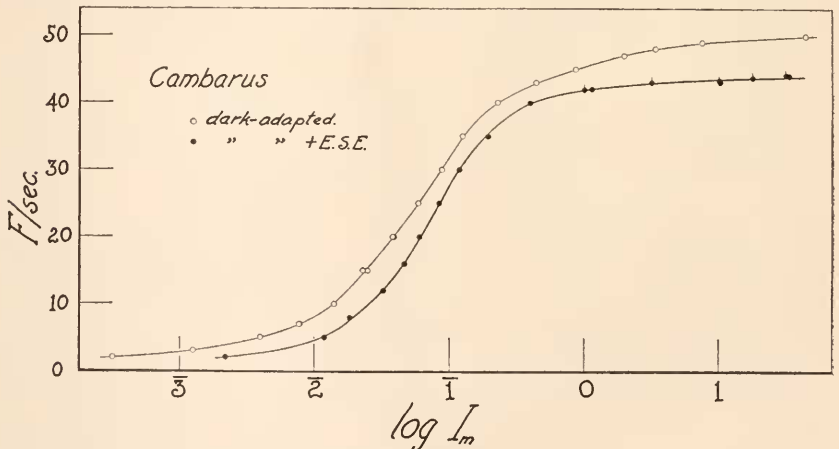


FIG. 2. $F - \log I$ curves for dark-adapted *Cambarus* and under the same conditions for individuals injected with eye-stalk extract (E.S.E.); Table I.

Figure 2 shows that the $F - \log I$ curve with *Cambarus* dark-adapted but under the influence of eye-stalk extract is moved toward higher intensities and exhibits a lower maximum. These are the

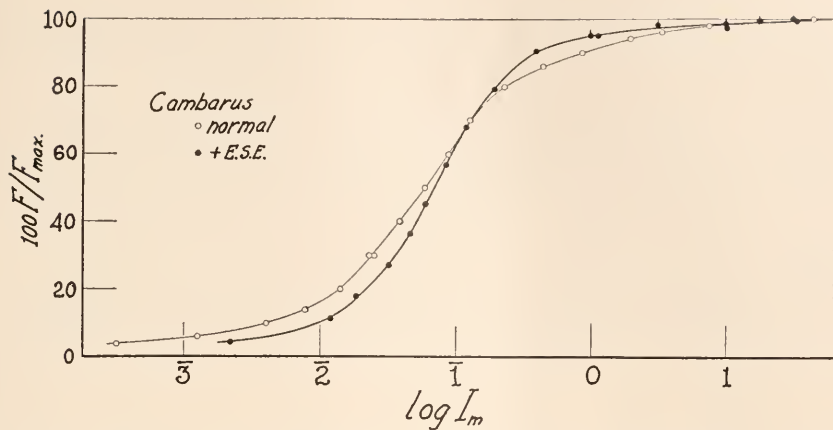


FIG. 3. The curves of Fig. 2 brought to the same $F_{max.}$ (= 100 per cent), to show change of shape.

results of a decrease in the total number of excitable elements (Crozier and Wolf, 1937c, 1938b), as expected.

The asymmetry of the curve is also decreased (Fig. 3). The

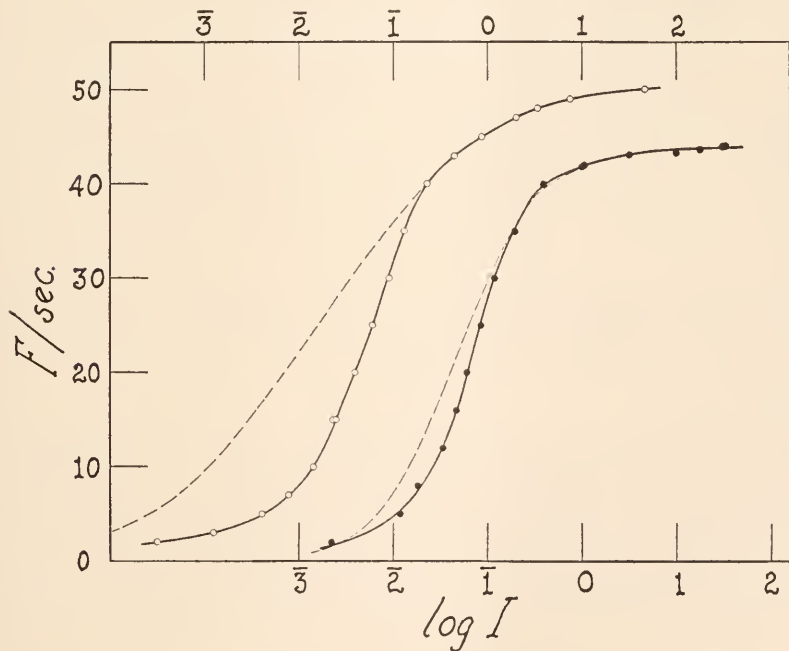


FIG. 4. The curves of Fig. 2 with probability integrals adjusted to the upper portions (cf. Crozier and Wolf, 1937c, 1938b, 1939, and paper in press), to show that the flicker-response contour after injection of eye-stalk extract departs less than the normal; see text.

sheathing of the ommatidia by pigment materially reduces the chance of photic action on additional elements as intensity increases, hence the slope of the $F - \log I$ curve cannot increase so rapidly.

It is to be presumed that in the absence of comparatively free passage of light through the eye (as in the dark-adapted state), the actual intensity at each receptor locus will be decreased. This cannot be a major factor in the changes shown in Fig. 2, else increase of intensity would find the $F - \log I$ curve continuously rising at its upper end.

The diffusion of light within the substance of the eye cannot be ignored, however. Figure 4 shows that the asymmetry of the flicker-

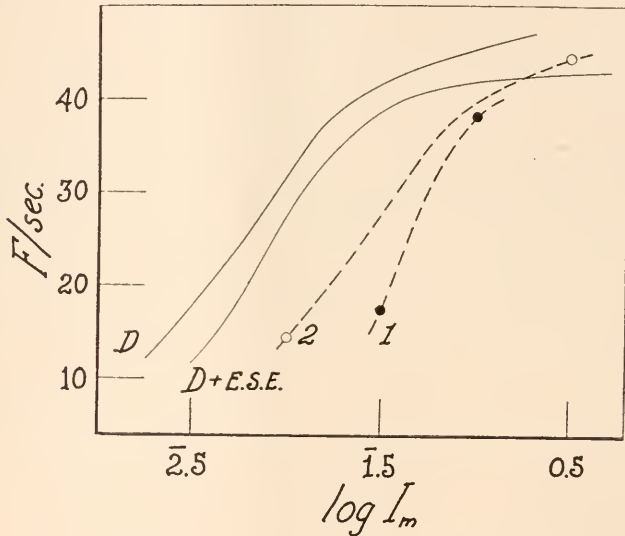


FIG. 5. Portions of the curves in Fig. 2 for dark-adapted *Cambarus* with ($D + E.S.E.$) and without (D) injection of eyestalk extract, and the first (1) and second (?) sets of readings (Table II) during the progress of dark adaptation after light adaptation.

response contour has been decreased (*cf.* Fig. 3), but not abolished. In view of the proximal movement of the distal retinal pigment under the influence of eye-stalk extract (Kleinholz, 1934, 1936, 1938; Welsh, 1939), this is not surprising. It probably explains the slight but detectable rise of the curve at the highest intensities used (Table I; Fig. 2), particularly when F_m is determined at constant flash-intensity; this cannot be accounted for by light adaptation (§ IV).

With allowance for this effect, a reasonable adjustment of an ideal probability integral can be made to the upper part of the curve (Fig. 4).

Comparison with the normal, in the same figure, shows that $\sigma'_{\log I}$ is, as expected, much reduced.

IV

Light adaptation of *Cambarus* reduces the $F - \log I$ curve (Table II, Fig. 5); with even brief residence in darkness the curve rises toward

TABLE II

Critical flash-frequencies at two flash-intensities for *Cambarus*: (1) very shortly after light adaptation to bright daylight; (2) after *ca.* 10 minutes in darkness; 3 observations on each of the same 4 individuals at all points; $21^\circ.5$, $t_L = t_D$. See text, and Fig. 4.

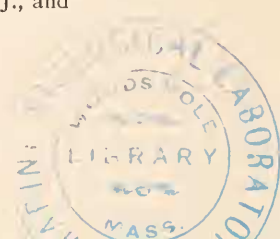
	$\log I$	F_m	P.E. $_{F_1}$
(1)	$\bar{1}.50$	17.5	0.491
	0.00	38.3	0.371
(2)	$\bar{1}.00$	14.5	1.52
	0.50	44.5	4.81

the position typical for dark adaptation (Fig. 5). Obviously, for a quantitative investigation of the kinetics of photic excitation, the effect of the migration of retinal pigment as governed by light and darkness must be ruled out. The present data supply the first evidence of a functional rôle of the position of the retinal pigment in matters of visual response. The result of light adaptation, as with certain other forms, is to reduce F at fixed I , but to follow by this means the recovery of excitability during subsequent darkness is made difficult by the fact that the retinal pigment also changes position. Either the pigment must be held in a fixed position throughout, by suitable repeated injection of eye-stalk extract, or else a procedure found for extrapolation to a constant condition of the pigment.¹ The latter could perhaps be achieved by determining the relation between the position of the $F - \log I$ curve and various known positions of the pigment; in any event the whole course of the function must be known.

SUMMARY

Injection of *Cambarus bartoni* with extract of eyestalks of this species forces migration of retinal pigments of individuals kept in darkness into positions characteristic of the light-adapted eye. In this condition the receptor elements of each ommatidium are effectively shielded from light passing through their neighbors. The flicker-response contour then differs in four particulars from that found when the retinal pigment is in the "dark" position, for which effective screen-

¹ For a somewhat analogous case of changing sensitivity during the interval of observation, a technic of this kind was used with *Agriolimax* (Crozier, W. J., and Wolf, E., 1928-29, *Jour. Gen. Physiol.*, 12: 83).



ing of the ommatidia is not present: F_{max} . is lowered; the whole curve is moved to higher intensities; the spread of the log I thresholds for the cumulative population of sensory effects is lessened; and the asymmetry of the $F - \log I$ curve is markedly reduced. It is pointed out that these results are to be expected if the asymmetry of the curve in normal dark-adaptation is due to the relation between flash-intensity and the curvature of the optic surface and divergence of the ommatidial axes.

CITATIONS

- BERNHARDS, H., 1916. *Zeitschr. f. wiss. Zool.*, **116**: 649.
 CROZIER, W. J., AND E. WOLF, 1939. *Jour. Gen. Physiol.*, **22**: 451.
 CROZIER, W. J., AND E. WOLF. *Jour. Gen. Physiol.*, in press (Cambarus, I).
 CROZIER, W. J., E. WOLF, AND G. ZERRAHN-WOLF, 1937a. *Proc. Nat. Acad. Sci.*, **23**: 516.
 CROZIER, W. J., E. WOLF, AND G. ZERRAHN-WOLF, 1937b. *Jour. Gen. Physiol.*, **21**: 17, 203.
 CROZIER, W. J., E. WOLF, AND G. ZERRAHN-WOLF, 1937c. *Jour. Gen. Physiol.*, **21**: 223.
 CROZIER, W. J., E. WOLF, AND G. ZERRAHN-WOLF, 1938a. *Proc. Nat. Acad. Sci.*, **24**: 125.
 CROZIER, W. J., E. WOLF, AND G. ZERRAHN-WOLF, 1938b. *Jour. Gen. Physiol.*, **21**: 463.
 DAY, E. C., 1911. *Bull. Museum Compar. Zoöl.*, **53**: 305.
 EXNER, S., 1891. *Die Physiologie der facettierten Augen von Krebsen und Insekten.* Leipzig u. Wien, 206 pp.
 KLEINHOLZ, L. H., 1934. *Proc. Nat. Acad. Sci.*, **20**: 659.
 KLEINHOLZ, L. H., 1936. *Biol. Bull.*, **70**: 159.
 KLEINHOLZ, L. H., 1938. *Biol. Bull.*, **75**: 510.
 PARKER, G. H., 1932. *Ergebn. Biol.*, **9**: 239.
 WELSH, J. H., 1939. *Biol. Bull.*, **77**: 119.